

Phylogeny and Evolution of the Division Procrustimorphi (Coleoptera, Carabidae) of the World as Deduced from the Mitochondrial ND5 Gene Sequences

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Abstract The division Procrustimorphi is the largest group in the carabine ground beetles. We have analysed the mitochondrial ND5 gene sequences of 236 specimens consisting of 123 species of the representative genera of this division. A molecular phylogenetic tree suggests that the Procrustimorphi is roughly divided into five geographically linked phylogenetic lineages, i.e., the European, the Caucasian, the Eurasian, the Tianshanese and the Chinese lineages. The detailed phylogenetic trees for each lineage are presented with discussions on the phylogenetic relationships between taxonomically defined taxa, and the mode of morphological differentiation in evolution of the ground beetles in this division.

Introduction

The division Procrustimorphi is the largest taxonomic group among nine (sub)divisions of the subtribe Carabina (see IMURA, 1996; IMURA *et al.*, 1998), containing nearly a half of the genera and 40% of the species in this subtribe. The carabid beetles of this division reveal the most remarkable morphological diversification and have been classified into 53–58 (sub)genera, over 300 species and many subspecies (IMURA & MIZUSAWA, 1996; BŘEZINA, 1999). Such a complexity of external morphology and yet a poor differentiation of the male genitalia make it difficult to estimate the phylogenetic relationships in the Procrustimorphi by cladistic analysis that depends on morphology alone.

In the present study, as a part of the research project on phylogeny and evolution of the carabid ground beetles, we have analysed the mitochondrial NADH dehydrogenase subunit 5 (ND5) gene sequences of about 236 specimens consisting of 123 species of the representative genera of this division collected from nearly the whole

distributional ranges of the world. They are roughly divided into five geographically linked phylogenetic lineages, namely, the European, the Caucasian, the Eurasian, the Tianshanese and the Chinese lineages. No cross contaminations of any genus within a given lineage to other lineages have been found. The phylogenetic relationships and the mode of morphological differentiation in evolution of the ground beetles in this division are discussed. The scientific names used in this study essentially follow IMURA and MIZUSAWA (1996).

Materials and Methods

Sampling

The specimens of procrustimorphous ground beetles used in this study were collected from most of the distributional ranges of this group in the world (Table 1). To prevent degradation of DNA, they were immediately killed and stored in 99% ethanol until use. A single individual of each species or subspecies was used for DNA extraction. The total DNA was extracted from thorax muscle (10–25 mg) by proteinase K digestion, phenol/chloroform extraction, and ethanol precipitation (SU *et al.*, 1996 a). Each DNA sample was dissolved in 100 μ l TE buffer (10 mM Tris, 1 mM EDTA, PH 8.0). Some of them used the QIAamp Tissue Kit (QIAGEN GmbH, Germany) (KIM *et al.*, 2000).

Amplification and sequencing

A region containing majority of the mitochondrial ND5 gene (1,083 bp) was amplified from the total DNA by the polymerase chain reaction (PCR) (SAIKI *et al.*, 1988) using the following primer pair: V1.06-1 (5'-CCT GTT TCT GCT TTA GTT CA-3') and V1.04-4 (5'-GTC ATA CTC TAA ATA TAA GCT A-3') (SU *et al.*, 1996 a). Amplification was carried out in 100 μ l reactions containing 10 μ l of the 10x Ex Taq Buffer (TaKaRa), 0.2 mM of each dNTP, 5 units of Ex Taq polymerase (TaKaRa), and 100 pmol of each primer. PCR was performed for 50 cycles, and each cycle consisted of denaturation at 94°C for 1 min, annealing at 50°C for 1 min and extension at 70°C for 2 min. The double-strand DNA fragment containing a 1,069-bp 3'-region of the ND5 gene, 7-bp of non-coding sequence and 7-bp 5'-terminus of the phe-tRNA gene was purified by electrophoresis in agarose gel (SU *et al.*, 1996 a).

Direct sequencing was performed by an automated ABI PRISM 377 DNA sequencer using the dideoxy chain-termination method (SANGER *et al.*, 1977). The reaction mixture for cycle sequencing consisted of 6 μ l of dRhodamine terminator cycle sequencing Ready Reaction with AmpliTaq-DNA Polymerase, FS (Applied Biosystems, Foster City, Calif.), 0.1–0.3 pmol/ μ l of DNA, 2.4 μ l (1 pmol/ μ l) of sequencing primer, and distilled water to a total volume of 15 μ l. The cycle-sequencing conditions were 25 cycles of 96°C for 10 sec, 50°C for 5 sec and 60°C for 4 min, and an indefinite hold at 4°C using a GeneAmp PCR system 9600 (Perkin Elmer). The DNA product was cleaned with Centri-Sep spin columns (Applied Biosystems) and vacuum-dried

before applying. Mostly, the two primers used for PCR were sufficient to read 1,083 bp sequence. In some case, the following internal primers were used.

Forward primers;

Ezo-2; 5'-TTC ATC TTT TAA CTC ATG CA-3'
 98p-330; 5'-TTT CTG TAA GAT GAA TTT C-3'
 HN-2; 5'-TTC ATC TTT TAA CTC ATG C-3'
 AI-2; 5'-TTC ATT TAT TAA CTC ATG C-3'
 K6-2; 5'-TCC TTC AAC ATT AGT TAC TG-3'
 A6-2; 5'-TCT TCT ACT TTA GTA ACA GC-3'
 6-3; 5'-TTC TTC TAC ATT AGT TAC AG-3'
 LC3-2; 5'-TTC ACC TTT TAA CTC ATG CA-3'
 Rc4-4; 5'-GAT CAA AAT TGA AAT GAA T-3'
 Rc4-5; 5'-GGT GCA TGA GTA GGG TAT G-3'.

Reverse primers;

AO-3; 5'-ATA TTC ATT TCA ACC TTG ATC-3'
 RcE-2; 5'-TGC ATC AGT TAA AAG ATG AA-3',
 AIRc-2; 5'-GCA TGA GTT AAT AAA TGA A-3'
 TARc-3; 5'-GAT CAG GGA TGA AAT GAA TAT-3'
 TARc-2; 5'-GCA TGA GTC AAA AGA TGA A-3'
 MRc-2; 5'-ATG AAC TAT AAG ATT TCC-3'
 ARc4-5; 5'-GGA GCA TGA ATT GGT TAT G-3'
 Lc3RC-2; 5'-TGC ATG AGT TAA AAG GTG A-3'
 WGRC-3; 5'-TTG ATC AAG GAT GGA ATG-3'
 HMK-3; 5'-ATA TTC ATT CCA TCC TTG ATC-3'
 CDA-3; 5'-ATA TTC ATT TCA TCC TTG ATC-3'.

The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL and GenBank nucleotide sequence databases with the accession numbers shown in Table 1.

Phylogenetic analysis

All alignments were carried out using the multiple alignment program CLUSTAL W, version 1.5 (THOMPSON *et al.*, 1994). The DNA sequence analysis package SINCA version 3.0 (Fujitsu System Engineering, Japan) was used for constructing the neighbor-joining (NJ) phylogenetic tree (SAITOU & NEI, 1987) with the evolutionary distances computed by KIMURA's two-parameter method (KIMURA, 1980). The trees were tested by 500 bootstrap replications.

The dating was done assuming that a 0.01 unit corresponds to 3.6 million years for the carabid ND5 gene according to SU *et al.* (1998, 2001).

Table 1 (on pp. 266–269). List of the specimens used in this study.

Specimen No.	Scientific name	Locality	DDBJ/EMBL/GenBank Accession No.
European lineage (Fig. 3)			
1	<i>Rhabdotocarabus melancholicus costatus</i>	Pontevedra, Serra Do Suido, Pigarzos, NW. Spain	AB101007
2	<i>Ctenocarabus galicianus galicianus</i>	Pontevedra, Serra Do Suido, Pigarzos, NW. Spain	AB101008
3	<i>C. g. galicianus</i>	La Coruna, Couto de Checo, NW. Spain	AB101009
4	<i>Cathoplius asperatus stenocephalus</i>	Agadir, S. Morocco	AB101010
5	<i>C. a. stenocephalus</i>	Agadir, S. Morocco	AB101011
6	<i>Macrothorax morbillosus constantinus</i>	Tunisia	AB101012
7	<i>M. m. constantinus</i>	Proto Cervo 50m, Is. Sardinia, Italy	AB101013
8	<i>Chrysocarabus auronitens auronitens</i>	Ranspringe, Hildesheim, Niedersachsen, N. Germany	AB101014
9	<i>C. a. auronitens</i>	Burgundy, E. France	D86205*
10	<i>C. a. auronitens</i>	Neustift-Neder, Stubaital, Innsbruck, Tirol, Austria	AB101015
11	<i>C. hispanus hispanus</i>	Correze, Brive-Correze, S. France	AB101016
12	<i>C. rutilans rutilans</i>	Corsavy, Pyrenees-Orientales, S. France	AB101017
13	<i>Imaius baronii baronii</i>	Shogran, Kaghan Valley, Mansehra, N. Pakistan	AB101018
14	<i>I. pachtouni pachtouni</i>	Utrot (Swat), 2,600m, Pakistan	AB101019
15	<i>Chrysocarabus olympiae olympiae</i>	Varallo, Piemowte, NW. Italy	AB101020
16	<i>Sphodristocarabus macrogonus macrogonus</i>	Ordu, NE. Turkey	AB101021
17	<i>S. m. macrogonus</i>	South of Ordu, NE. Turkey	AB101022
18	<i>S. m. macrogonus</i>	Zigana Pass, 1,900m, South of Trabzon, NE. Turkey	AB101023
19	<i>S. m. macrogonus</i>	Kumbet Vil., Giresun, NE. Turkey	AB101024
20	<i>S. bohemanii ssp.</i>	South of Ramsar, 1,700m, Chorteh, Mazandaran, N. Iran	AB101025
21	<i>S. armeniacus armeniacus</i>	Karcag Dag, NE. Turkey	AB101026
22	<i>S. adamsi subcaneus</i>	Mt. Tepli, 2,700-3,200m, Sources of Riv. Liadon, Bokovoy Mt. R., Osetia, C. Caucasus, Russia	AB101027
23	<i>S. rotundicollis</i>	Tscharnali Vil., South of Batumi, Adzharia, SW. Caucasus, Russia	AB101028
24	<i>S. adamsi janthinus</i>	Kluish, 400m, Goriashiy, W. Caucasus, Russia	AB101029
25	<i>S. a. janthinus</i>	Lazarevskoye, Sochi, W. Caucasus, Russia	AB101030
26	<i>S. heinzi heinzi</i>	West of Giresun, NE. Turkey	AB101031
27	<i>S. h. heinzi</i>	Giresun, NE. Turkey	AB101032
28	<i>S. sovitzki otingensis</i>	Karcag Dag, NE. Turkey	AB101033
29, 30	<i>S. s. otingensis</i>	Karcag Dag, NE. Turkey	AB101034
Caucasian lineage (Fig. 4)			
31	<i>Cechnochilus heydenianus heydenianus</i>	Mt. Pshekhassu, W. Caucasus, Russia	AB101035
32	<i>C. h. heydenianus</i>	Mt. Aibga, W. Caucasus, Russia	AB101036
33	<i>C. h. heydenianus</i>	Mts. Turinye, Aibga, W. Caucasus, Russia	AB101037
34-36	<i>C. boeberi tschitscherini</i>	Mt. Khumaratkchok, 2,300m, Skalystyi Mt. R., Osetia, C. Caucasus, Russia	AB101038
37	<i>Microplectes convallium convallium</i>	Sochi, W. Caucasus, Russia	AB101039
38	<i>M. c. convallium</i>	Mt. Messazhai, North of Tuapse, W. Caucasus, Russia	AB101040
39	<i>M. c. convallium</i>	Lazarevskoye, Sochi, W. Caucasus, Russia	AB101041
40	<i>M. c. convallium</i>	Ashe R., Shaukai, Sochi, W. Caucasus, Russia	AB101042
41	<i>M. c. argonautarum</i>	Southern slope of Aibga Mt. R., 2,100m, W. Caucasus, Russia	AB101043
42	<i>M. c. argonautarum</i>	Fagus, Krasnaya, Galion, Aibga, W. Caucasus, Russia	AB101044
43	<i>Archiplectes starcki starcki</i>	Western part of Aibga subalps, 1,700m, W. Caucasus, Russia	AB101045
44	<i>A. reitteri ssp.</i>	Retshka Valley, 1,200m, Tikhaya, Mzymta, Aibga, W. Caucasus, Russia	AB101046
45	<i>A. r. ssp.</i>	Mzymta Valley 1,200m, Aibga, W. Caucasus, Russia	AB101047
46, 47	<i>Tribax osseticus osseticus</i>	Mt. Tepli, 2,700-3,200m, Sources of Riv. Liadon, Bokovoy Mt. R., Osetia, C. Caucasus, Russia	AB101048
48	<i>Archiplectes starckianus theseus</i>	Mt. Messazhai, North of Tuapse, W. Caucasus, Russia	AB101049
49	<i>A. s. starckianus</i>	Abissinia, Lazarevskoye, Sochi, W. Caucasus, Russia	AB101050
50	<i>A. s. starckianus</i>	Svirskaya Gorge, Lazarevskoye, Sochi, W. Caucasus, Russia	AB101051
51	<i>A. starcki starcki</i>	N. slope of Mt. Pshekhassu, W. Caucasus, Russia	AB101052
52	<i>Ar. starcki starcki</i>	S. slope of Aibga, 2,100-2,200m, W. Caucasus, Russia	AB101053
53	<i>Tribax kasbekianus ssp.</i>	Mt. Khumaratkchok, 2,300m, Skalystyi, Osetia, C. Caucasus, Russia	AB101054
54	<i>T. k. ssp.</i>	Mt. Tepli, 2,700-3,200m, Sources of Riv. Liadon, Bokovoy Mt. R., Osetia, C. Caucasus, Russia	AB101055
55	<i>T. titan ssp.</i>	Mt. Messazhai, North of Tuapse, W. Caucasus, Russia	AB101056
56	<i>T. agnatus agnatus</i>	Turinye Mts., 2,300m, Aibga, W. Caucasus, Russia	AB101057
57	<i>T. a. agnatus</i>	Mt. Aibga, 2,400m, W. Caucasus, Russia	AB101058
58	<i>T. a. agnatus</i>	Kamennyi Stoib, 2,200m, Aibga, W. Caucasus, Russia	AB101058
59	<i>T. circassicus circassicus</i>	N. slope of Mt. Pshekhassu, W. Caucasus, Russia	AB101059
60	<i>T. c. circassicus</i>	Western part of Aibga subalps, 1,700m, W. Caucasus, Russia	AB101060
61	<i>T. puschkini ponticus</i>	Giresun, NE. Turkey	AB101061
62	<i>T. p. ponticus</i>	Zigana Pass, 1,900m, South of Trabzon, NE. Turkey	AB101062
63	<i>T. p. ishikawianus</i>	Karcag Dag, NE. Turkey	AB101063
64	<i>T. p. ishikawianus</i>	Cankurtaran Pass, 700m, Hopa, NE. Turkey	AB101064
65	<i>T. p. zyzysus</i>	Artvin, NE. Turkey	AB101065
Eurasian lineage (Fig. 5)			
66	<i>Megodontus exaratus exaratus</i>	Mt. Skalystyi, Osetia, C. Caucasus, Russia	AB101066
67	<i>M. septemcarinatus septemcarinatus</i>	Riv. Psezuapse, Sochi, W. Caucasus, Russia	AB101067
68	<i>M. s. septemcarinatus</i>	Mt. Skhapatch, Sochi, W. Caucasus, Russia	AB101068
69	<i>M. imperialis imperialis</i>	Putintzevo Vil., 600m, Zyrianovsk, E. Kazakhstan	AB101069

Table 1. (Continued).

70	<i>M. schoenherri sajanus</i>	Cheremushki, 800m, Sajnogorsk, Krasnojarski, Russia	AB101070
71	<i>M. s. schoenherri</i>	Pervukha, 500m, S. Ural, Russia	AB101071
72	<i>M. s. schoenherri</i>	Mun Lake Bass, 850m, Tuva, Russia	AB101072
73	<i>M. vietinghoffi bowringi</i>	Amur, Russia	AB101073
74	<i>M. v. leptoglyptus</i>	Sakhalin, Russia	AB101074
75	<i>M. kolbei aino</i>	Kamikawa, Hokkaido, Japan	D50365*
76	<i>M. leachi panzeri</i>	Putintzevo Vil., 600m, Zyrianovsk, Kazakhstan	AB101075
77	<i>M. ermaki ermaki</i>	Oiskoe Lake, 2,200m, Khakassia, Russia	AB101076
78	<i>M. violaceus purpurascens</i>	West of Ramsel, East of Lingen, N. Germany	AB101077
79	<i>M. v. purpurascens~violaceus</i>	North of Neudersun, West of Dorpen, Emsland, N. Germany	AB101078
80	<i>M. v. piceus</i>	Campo Imperatore, Gran Sasso, Abruzzi, Italy	AB101079
81	<i>M. v. violaceus</i>	Surwold, Emsland, N. Germany	AB101080
82	<i>M. v. violaceus</i>	Trencin, Slovakia	AB101081
83	<i>M. v. violaceus</i>	Mala Fatra Park, Zilina, Slovakia	AB101082
84	<i>M. v. violaceus</i>	Tatraska Lomnica, Tatra Park, Slovakia	AB101083
85	<i>M. v. violaceus</i>	Epleny, Zirc Veszprem, W. Hungary	AB101084
86	<i>M. germarii savinicus</i>	Miane Campea, Veneto, NE. Italy	AB101085
87	<i>M. g. savinicus</i>	Cison di Valmarino, Veneto, NE. Italy	AB101085
88	<i>Procerus gigas gigas</i>	Mt. Brkini Mislice, Slovenija	AB101086
89	<i>P. scabrosus caucasicus</i>	Mt. Beshtau, Stavropol, C. Caucasus, Russia	AB101087
90	<i>P. s. audouini</i>	Voc Muezzinler, Adapazaripr, NW. Turkey	AB101088
91	<i>P. s. audouini</i>	Dereli Valley, Giresun, NE. Turkey	AB101089
92	<i>P. s. audouini</i>	Trabzon, NE. Turkey	AB101090
93	<i>P. s. sommeri</i>	Bursa, Turkey	AB101090
94	<i>Megodontus bonvouloiri bonvouloiri</i>	Kumbet Vil., 1,100m, South of Giresun, NE. Turkey	AB101091
95	<i>M. b. bonvouloiri</i>	Zigana Pass, 1,900m, Trabzon, NE. Turkey	AB101092
96	<i>M. stroganowi ssp.</i>	Bostanabad (Waldzone), 1,200m, Piri, Gilan, N. Iran	AB101093
97	<i>M. persianus persianus</i>	Dar-e-Dasht, 1,000m, Gilan, N. Iran	AB101094
98	<i>M. p. transfugus</i>	South of Ramsar, 1,700m, Chorteh, Mazandaran, N. Iran	AB101095
99	<i>M. p. morgani</i>	Chorteh, 500m, Mazandaran, N. Iran	AB101096
100	<i>Procrustes chevrolati internatus</i>	NW. Turkey	AB101097
101	<i>P. c. internatus</i>	Abant, NW. Turkey	AB101098
102	<i>P. c. thirki</i>	Giresun, NE. Turkey	AB101099
103	<i>P. c. thirki</i>	Ordu, NE. Turkey	AB101100
104	<i>P. c. thirki</i>	Mercan, N. Turkey	AB101101
105	<i>Lamprostus punctatus punctatus</i>	Topbogazi Pass, 800m, Iskenderun, SE. Turkey	AB101102
106	<i>L. p. punctatus</i>	Topbogazi Pass, 800m, Iskenderun, SE. Turkey	AB101103
107	<i>Procrustes coriaceus coriaceus</i>	Tatra National Park, High Tatras, Slovakia	AB101104
108	<i>P. c. coriaceus</i>	Guardabosone, Piemonte, NW. Italy	AB101105
109	<i>P. c. coriaceus</i>	Veneto, Miane, Italy	AB101106
110	<i>P. c. coriaceus</i>	Epleny, Zirc Veszprem, W. Hungary	AB101107
111	<i>P. c. coriaceus</i>	North of Bramsche, North of Osnebruck, N. Germany	AB101108
112	<i>P. c. coriaceus</i>	Stubaital, Innsbruck, Tirol, Austria	AB101109
113	<i>P. c. coriaceus</i>	Mala Fatra National Park, Zilina, C. Slovakia	AB101110
114	<i>P. t. talyshensis talyshensis</i>	Masuleh, 1,700m, Gilan, N. Iran	AB101111
115	<i>P. t. talyshensis</i>	Rudbarak, 1,600m, Mazandaran, N. Iran	AB101112
116	<i>Lamprostus prasinus prasinus</i>	Rostamabad, 1,700m, Piri, Gilan, N. Iran	AB101113
117	<i>L. torosus giresuni</i>	Giresun, NE. Turkey	AB101114
118	<i>L. chalconatus chalconatus</i>	Mercan, C. Turkey	AB101115
119	<i>L. c. chalconatus</i>	Spikor, CE. Turkey	AB101116
120	<i>L. c. chalconatus</i>	Umg. Tufanbeyli, 1,500m, Adana, Turkey	AB101117
121	<i>L. torosus spinolae</i>	Corum, Turkey	AB101118
122	<i>L. t. rabaroni</i>	Mesudiye, NE. Turkey	AB101119
123	<i>L. t. giresuni</i>	Giresun, NE. Turkey	AB101120
124	<i>L. nordmanni ducalis</i>	Zigana Pass, 1,900m, South of Tranzon, NE. Turkey	AB101121
125	<i>L. n. pseudorobustus</i>	Kumbet Vil., 1,100m, South of Giresun, N. Turkey	AB101122
126	<i>L. erenleriensis erenleriensis</i>	Abant, NW. Turkey	AB101123
127	<i>L. torosus ssp.</i>	Kiziloren, 1,600m, Konya, Turkey	AB101124
128	<i>Oxycharabus saphyrinus notabilis</i>	Tahtakopru, 1,300m, Pass between Domanic & Bursa, Turkey	AB101125
129	<i>O. s. pesudosaphyrinus</i>	Abant, NW. Turkey	AB101126
130	<i>Procrustes piochardi praestigiator</i>	Aaramoun near Damour, Lebanon	AB101127
131	<i>P. p. moravitzii</i>	Qal'at Samaan, ca. 400m, 30km Northwest of Aleppo, NW. Syria	AB101128
132	<i>Lamprostus syrus ?</i>	Northwest of Irbid, 100m, Al Himma, Jordan	AB101129
133	<i>Procrustes mulsantianus ssp.</i>	Topbogazi Pass, 800m, Iskenderun, SE. Turkey	AB101130
134	<i>P. m. ssp.</i>	Topbogazi Pass, 800m, Iskenderun, SE. Turkey	AB101131
135	<i>Lamprostus hemprichi ssp.</i>	Aaramoun near Damour, Lebanon	AB101132
136	<i>Procrustes impressus ssp.</i>	West of AD Dana, 300m, Aleppo, NW. Syria	AB101133
137	<i>P. mulsantianus bernhauerorum</i>	2km South of Feke, 600m, Adana, Turkey	AB101134
138	<i>Lamprostus punctatus antakya</i>	Enek Vil., Pass ca. 500m, Antakya, SE. Turkey	AB101135
Tianshanese lineage (Fig. 6)			
139	<i>Cratophyrtus kaufmanni microcratophyrtus</i>	Kashka-Suu Riv, 2,700-3,400m, Eastern slope of Koek-Ala Mts., S. Slope of Tshatkal Mts., Kirghizia	AB101136
140	<i>Pantophyrtus brachypedilus evstigneevi</i>	15km South of Kozuchak, 600m, Talas, Kirghizia	AB101137
141	<i>P. turcomannorum longipedatus</i>	5-10km East of Britshnulla, 900-1,000m, Koksuv Riv., W. Tianshan, Uzbekistan	AB101138
142	<i>P. brachypedilus brachypedilus</i>	20km ENE. of Britshnulla, 1,300m, Koksuv Riv., W. Tian-Shan, Uzbekistan	AB101139
143	<i>Eotribax hiekei hiekei</i>	Zailiyskij Alatau, Kirghizia	AB101140
144	<i>Cratocarabus jacobsoni jacobsoni</i>	Alma-Atinka Riv., 2,500m, Zailiyskij Alatau, SE. Kazakhstan	AB101141

Table 1. (Continued).

145	<i>Cratocheenus akinini elisabethae</i>	Alma-Atinka Riv., 2,500m, Zailiyskij Alatau, SE. Kazakhstan	AB101142
146	<i>C. jacobsoni jacobsoni</i>	Zailiyskij Alatau, Kazakhstan	AB101143
147	<i>Cratocheenus akinini</i> ssp.	Kara-Kichi-Kudzhur, Dolon Pass, 2,700m, Kirghizia	AB101144
148	<i>C. a. ketmenensis</i>	Tuiuk, 2,000m, Ketmen Mts., Kazakhstan	AB101145
149	<i>C. a. puellus</i>	Mts. Inyltshek, Kirghizia	AB101146
150	<i>C. a. puellus</i>	near Tashkoro, along Kaindy Riv., Kirghizia	AB101147
151	<i>C. a. puellus</i>	Kok-Kiya Val., 3,000m, Turgen Riv., Terskei Mts., Kirghizia	AB101148
152	<i>C. a. puellus</i>	Terskei Alatau, Kirghizia	AB101149
153	<i>Cratocephalus cicatricosus cicatricosus</i>	Tuiuk, 2,000m, Ketmen Mts., Kazakhstan	AB101150
154	<i>C. c. cicatricosus</i>	Mt. Kok-Tiube, 1,000m, Almaty, Kazakhstan	AB101151
155	<i>C. c. cicatricosus</i>	Mt. Acatau, Zailiyskij Alatau, Kazakhstan	AB101152
156	<i>C. c. cicatricosus</i>	Transili Alatau, Kirghizia	AB101153
157	<i>C. c. corrugis</i>	Kara-Balta, 1,200m, Kirghizia	AB101154
158	<i>C. c. cicatricosus</i>	Southern slope of Dzhungarsky Alatau, Kazakhstan	AB101155
159	<i>Eotribax valikhanovi valikhanovi</i>	Mts. Inyltshek, Kirghizia	AB101156
160	<i>E. eous eous</i>	Terskei Alatau, Kirghizia	AB101157
161	<i>E. e. eous</i>	Sary-Moinok, 2,800m, Terskei Mts., Kirghizia	AB101158
162	<i>E. e. irae</i>	Suik Pass, 4,000m, Terskei Mts., Kirghizia	AB101159
163	<i>Deroplectes staudingeri staudingeri</i>	Turkestan Mts., Kirghizia	AB101160
164	<i>Ceohenotribax petri petri</i>	Tuiuk, 2,000m, Ketmen Mts., Kazakhstan	AB101161
165	<i>Cratocephalus balassogloi balassogloi</i>	Tuiuk, 2,000m, Ketmen Mts., Kazakhstan	AB101162
166	<i>C. solskyi solskyi</i>	Tuiuk, 2,000m, Ketmen Mts., Kazakhstan	AB101163
167	<i>C. s. toropovi</i>	20km North of Bakanas, 350m, Kazakhstan	AB101164
168	<i>Alipaster pupulus pupulus</i>	Terskei Alatau, Kirghizia	AB101165
169	<i>A. p. pupulus</i>	Kok-Kiya Val., 3000m, Turgen Riv., Terskei Mts., Kirghizia	AB101166
170	<i>A. p. pupulus</i>	Terskei Alatau, Kirghizia	AB101167
171	<i>Cratophytus kaufmanni kaufmanni</i>	Turkestan Mts., Kirghizia	AB101168
172	<i>C. k. kaufmanni</i>	Turkestan Mts., Kirghizia	AB101169
173	<i>Leptoplectes merzbacheri merzbacheri</i>	Inyltshek Mts., Kirghizia	AB101170
174	<i>Pantophytus turcomanorum karaalomicus</i>	upper course of Ak-Buura Riv., 2,800m, rt. trib. of Kalta-Boz Riv., Boz-Tektyr Riv., E. Alai, Kirghizia	AB101171
175	<i>P. t. karaalomicus</i>	Zagri, 3,000m, Mt. Kitshik-Alai, Kirghizia	AB101172
Chinese lineage (Fig. 7)			
176	<i>Pseudocoptolabrus taliensis liqiensis</i>	North of Sabde, 3,200m, W. Sichuan, China	AB101173
177	<i>P. burmanensis burmanensis</i>	Dingo-mai, near Putao, N. Myanmar	AB101174
178	<i>Megodontoides erwini erwini</i>	Wenchuan, C. Sichuan, China	AB101175
179	<i>Acathacius alexandrae idolon</i>	Wenxian, S. Gansu, China	AB050697*
180	<i>A. a. fanningi</i>	above Jiuzhaizhen, N. Sichuan, China	AB050698*
181	<i>A. a. fanningi</i>	above Jiuzhaizhen, N. Sichuan, China	AB050699*
182	<i>Coptolabrodes haeckeri haeckeri</i>	Mt. Taibai Shan, 3,000m, S. Shaanxi, China	AB050696*
183	<i>Imaioides businskyi businskyi</i>	Mt. Yulongxue Shan, NW. Yunnan, China	AB101176
184	<i>Lasiocoptolabrus sunwukong sunwukong</i>	Houzhenzi, 1,350m, Qinling Mts., Shaanxi, China	AB101177
185,186	<i>Cratocarbubus viridifossulatus viridifossulatus</i>	Fengtongzhai, Baoxing, C. Sichuan, China	AB010718*
187	<i>A. v. rizeanus</i>	Rizegou Val., Jiuzhaigou Xian, N. Sichuan, China	AB050687*
188	<i>A. v. rizeanus</i>	Primitive Forest, Jiuzhaigou, N. Sichuan, China	AB050677*
189	<i>A. v. businskyorum</i>	Mt. Shennongding, Dashennongjia, W. Hubei, China	AB101178
190	<i>A. v. ventrosior</i>	Pass Gonggaling, SW. Jiuzhaigou Xian, N. Sichuan, China	AB101179
191	<i>Shunichiocarbubus uenoiianus uenoiianus</i>	Shennongjia, W. Hubei, China	AB050688*
192	<i>Phagocarbubus crassesculptus qunqingicolar</i>	Mt. Tabai Shan, S. Shaanxi, China	AB101180
193	<i>P. c. crassesculptus</i>	Xiaolongmen, Beijing, China	AB010719*
194	<i>P. c. jollyi</i>	Wenxian, S. Gansu, China	AB050689*
195	<i>P. c. paradiptus</i>	North of Sabde, 3,200m, W. Sichuan, China	AB101181
196	<i>P. c. diruptus</i>	above Jiuzhaizhen, Jiuzhaigou Xian, N. Sichuan, China	AB050690*
197	<i>Neoplesius lama garzeicus</i>	South of Qagca, 4,000m, NW. Sichuan, China	AB101182
198	<i>Pseudocranion gansuense venerandum</i>	Tochizi, 2,300m, South of Wudu, S. Gansu, China	AB101183
199	<i>P. wenxianicola wenxianicola</i>	North of Wenxian, 3,000m, S. Gansu, China	AB101184
200	<i>P. benjamini meissonieri</i>	North of Wenxian, 3,000m, S. Gansu, China	AB101185
201	<i>P. sackeni gamisiense</i>	Jiuzhaigou, N. Sichuan, China	AB101186
202	<i>P. s. gamisiense</i>	Primitive Forest, Jiuzhaigou, N. Sichuan, China	AB101187
203	<i>Ecocoptolabrus exiguus fanianus</i>	Mt. Taibai Shan, S. Shaanxi, China	AB050691*
204	<i>Calocarbubus aristochroides aristochroides</i>	West of Maniganggo, 4,100m, NW. Sichuan, China	AB050693*
205	<i>C. a. qagcaensis</i>	South of Qagca, NW. Sichuan, China	AB050692*
206	<i>Neoplesius draco draco</i>	Mt. Jiuding Shan, 3,300m, NC. Sichuan, China	AB101188
207	<i>N. lama yajiangensis</i>	20km West of Yajiang, 4,300-4,600m, W. Sichuan, China	AB101188
208	<i>N. wagae alboequus</i>	Mt. Baimaxue shan, 35km South of Deqen, 4,300m, NW. Yunnan, China	AB101190
209	<i>N. w. wagae</i>	Margyang, Lhasa, Xizang, China	AB001515*
210	<i>Eoecechenus leptoplesioides leptoplesioides</i>	Markam, Xizang, China	AB001510*
211	<i>Neoplesius alpherakii alpherakii</i>	West of Kangding, W. Sichuan, China	AB101191
212	<i>Pseudocranion remondianumremondianum</i>	South of Jiuzhaigou Xian, N. Sichuan, China	AB101192
213	<i>Neoplesius kaschkarowi kaschkarowi</i>	Northeast of Qianning, W. Sichuan, China	AB101193
214	<i>N. nanschanicus nocticolor</i>	Pass Gonggaling, Southwest of Jiuzhaigou Xian, N. Sichuan, China	AB101194
215	<i>Damaster blaptoides blaptoides</i>	Nagayo, Nagasaki, W. Japan	AB013979*
216	<i>D. b. oxuroides</i>	Noda, Chiba, C. Japan	AB013945*
217	<i>D. b. babaianus</i>	Murayama, Yamagata, N. Japan	AB013931*
218	<i>Cephalornis potanini potanini</i>	Wenxian, S. Gansu, China	AB050694*
219	<i>C. p. remondorum</i>	Jiuzhaigou Xian, N. Sichuan, China	AB050695*
220	<i>Acoptolabrus gehinii aereicollis</i>	Urahoro, Hokkaido, N. Japan	D50339*

Table 1. (Continued).

221	<i>A. munakatai munakatai</i>	Mt. Shirakami, Hokkaido, N. Japan	AB041083*
222	<i>A. gehinii radiatocostatus</i>	Hiroo, Hokkaido, N. Japan	D50429*
223	<i>Eupachys glyptopterus glyptopterus</i>	Belozersk, Burjatia, E. Siberia, Russia	AB050728*
224	<i>Acoptolabus constricticollis constricticollis</i>	Nakhodka, Primorsky, Russia	AB050731*
225	<i>A. leechi yooni</i>	Odac-san Mts., S. Korea	AB041079*
226	<i>A. mirabilissimus mirabilissimus</i>	Odac-san Mts., S. Korea	AB050684*
227	<i>Shenocoptolabus osawai osawai</i>	Shennongjia, W. Hubei, China	AB050682*
228	<i>S. o. micangshanus</i>	Mt. Guangwu Shan, Nanjiang, NE. Sichuan, China	AB101195
229	<i>S. o. micangshanus</i>	Daba, Micangshan Mts., Nanjiang, NE. Sichuan, China	AB050681*
230	<i>Cathalicus brandti brandti</i>	Bagoulin, Haidian, Beijing, China	AB050686*
231	<i>Coptolabus jankowskii ssp.</i>	Mt. Palgong-san, S. Korea	AB101196
232	<i>C. formosus bousqueti</i>	Nanping (=Jiuzhaigou Xian), N. Sichuan, China	AB101197
233	<i>C. kubani kubani</i>	Daju, 1,900-2,000m, NW. Yunnan, China	AB101198
234	<i>C. ignimentallus guangxicola</i>	Mt. Dayao Shan, Guangxi, China	AB050685*
235	<i>C. smaragdinus monilifer</i>	Mt. Halla-san, Is. Cheju-do, S. Korea	AB101199
236	<i>C. s. branickii</i>	Chiri-san Mts., S. Korea	AB101200

* Taken from the previous studies (Imura *et al.*, 1997; 1998; Su *et al.*, 1996a; 1996b; 1996c; 1998; 2001; Tominaga *et al.*, 2000).

Results and Discussion

The following discussions have been made with an assumption that the ND5 phylogeny may be equated to the species phylogeny without considering a possible horizontal transfer of mitochondria, ancestral polymorphism and random lineage sorting. These factors should ultimately be examined to reach the final conclusion for each item.

The Procrustimorphi as a distinct phylogenetic and taxonomic group

A phylogenetic tree of the ND5 gene from the representative divisions of the Carabina reveals that all the species of the Procrustimorphi (except three; see below) are grouped together without any cross contamination by the species taxonomically belonging to other divisions, despite a short branch length with a low bootstrap value that supports the Procrustimorphi. The situation holds when replacement of outgroup species by others or addition/removal of the species. We interpret this tree as showing that the Procrustimorphi is one distinct phylogenetic and taxonomic group, and various phylogenetic lineages in this division emerged shortly after the radiation of the Carabina. The short branch length supporting the division on the tree would not have been a result of saturation of nucleotide substitutions, since the actual percentage of substitution is linearly proportional to *D*, the value corrected for multiple substitution by KIMURA's method (KIMURA, 1980).

Ctenocarabus (distributed in the Iberian Peninsula) plus *Rhabdotocarabus* (Iberian Peninsula and northern Africa) and *Cathoplius* (northern Africa) have been considered to be the members of the Procrustimorphi (cf. IMURA, 1996; IMURA & MIZUSAWA, 1996). However, the ND5 molecular phylogenetic tree suggests that these three (sub)genera are not clustered with other procrustimorphous members and they, above all the former two, most probably form distinct divisions independent from the Procrustimorphi. *Ctenocarabus* and *Rhabdotocarabus* form one cluster, although their divergence occurred a long time ago (Fig. 1). The branch length on the tree for *Cathoplius* is exceptionally shorter than those of others. This might be because of a slower rate of nucleotide substitutions. To settle their reliable phylogenetic positions, more ap-

propriate gene should be analysed.

Geographically linked phylogenetic lineages

Figure 1 shows a phylogenetic tree of the ND5 gene from the representative species of the Procrustimorphi. Only selected species are included in this tree among much more species analysed. As noted above, *Ctenocarabus*, *Rhabdotocarabus* and *Cathoplius* formed the clusters outside the Procrustimorphi, and they are considered as the outgroups. There exist at least 5 lineages, each of which is further divided into two to ten sublineages. Here also, the branch length supporting each lineage is short, and this is interpreted to show that diversification of different lineages occurred within a short time after the radiation of the Carabina. It is noteworthy that there is no case in that a given genus appears in two or more lineages. In other words, each lineage is composed of the genera specific to it, suggesting overall correctness of the tree. Another point of interest would be that each lineage is geographically linked as mentioned below. For the distributional range of each lineage, see Fig. 2.

1) The European lineage. This lineage is constructed by *Chrysocarabus*, *Macrothorax* (mainly distributed in Europe) and *Sphodristocarabus* (Asia Minor to northern Iran). The genus *Imaibius* is also placed here, although its distributional range (mainly known from Kashmir) is separated from those of other members in this division.

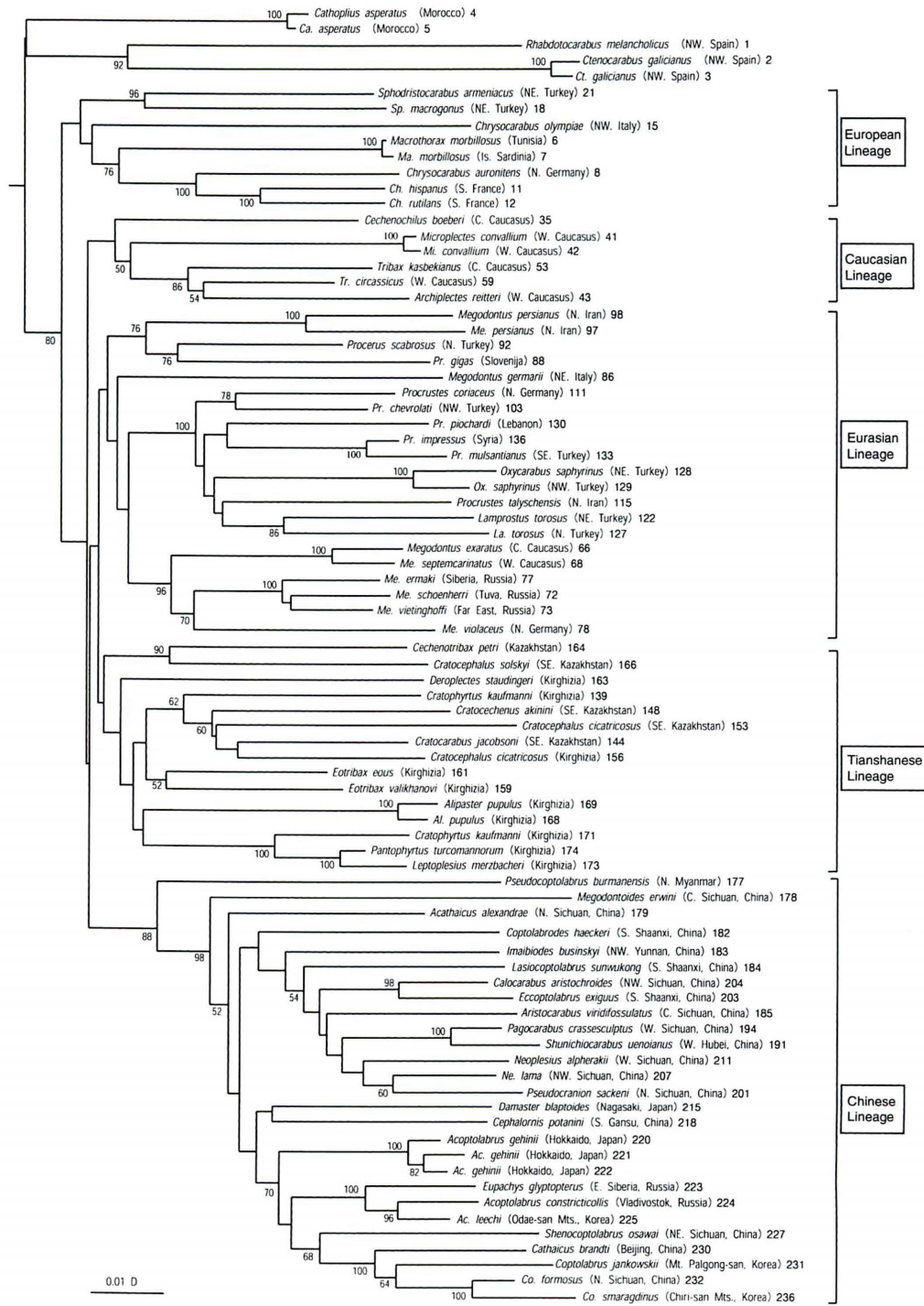
2) The Caucasian lineage. The genera *Microplectes*, *Cechenochilus*, *Archiplectes* and *Tribax* are placed here. All distributed in Caucasia and the nearby regions (northeastern Turkey, etc.).

3) The Eurasian lineage. *Megodontus*, *Procerus*, *Procrustes*, *Lamprostus* and *Oxycarabus* are included here. The distributional range of this lineage is exceptionally wide among the Procrustimorphi, covering the greater part of the northern Eurasia and northwestern North America.

4) The Tianshanese lineage. This lineage is composed of the genera *Cratophyrtus*, *Pantophyrtus*, *Eotribax*, *Cratocarabus*, *Cratocechenus*, *Deroplectes*, *Cechenotribax*, *Alipaster* and *Leptoplesius*, all distributed in the Tianshan Mountains of Central Asia.

5) The Chinese lineage. This lineage contains the following genera; *Pseudocoptolabrus*, *Megodontoides*, *Acathaicus*, *Coptolabrodes*, *Imaibiodes*, *Lasiocoptolabrus*,

Fig. 1. Phylogenetic tree (NJ) of the mitochondrial ND5 gene of the division Procrustimorphi. This tree was constructed by the representative species (marked with asterisks in Figs. 3–7) from each group. Throughout the trees in this paper, distance (D) denotes Kimura's two-parameter evolutionary distance (KIMURA, 1980). Value at the node represents bootstrap confidence level (%) based on 500 re-samplings. Numerals following the scientific name and locality correspond to the specimen numbers shown in Table 1. Most of the scientific names in this table include those for the genus and the species as well as the subspecies whatever possible. The specimen, for which identification of the subspecies was uncertain, is shown as "ssp." instead of the subspecific name after the specific name. In the phylogenetic trees in this paper, the subspecific name is given only when it is considered to be necessary for discussion.



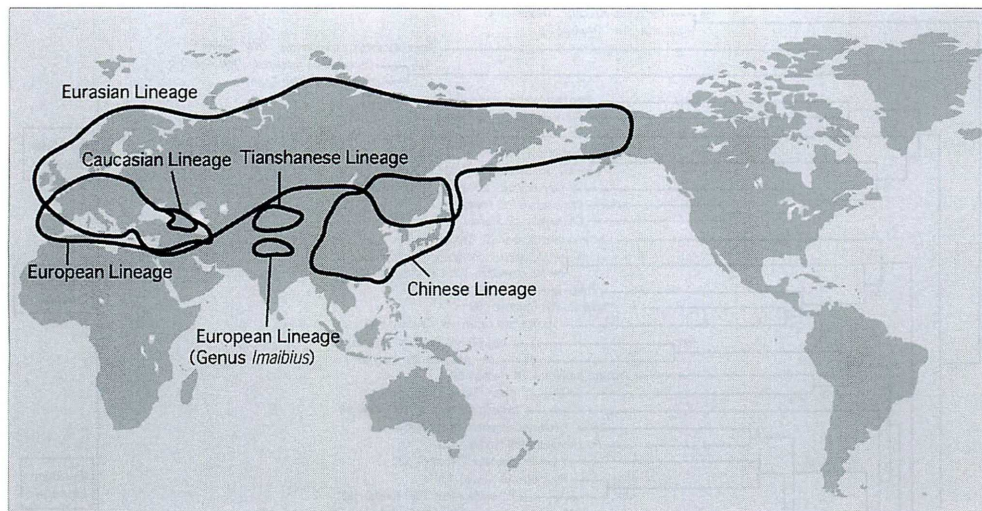


Fig. 2. Distributional ranges of each geographically linked group of the Procrustimorphi in the world.

Aristocarabus, *Shunichiocarabus*, *Pagocarabus*, *Neoplesius*, *Pseudocranion*, *Eccoptolabrus*, *Calocarabus*, *Eocechenus*, *Cupreocarabus*, *Damaster*, *Cephalornis*, *Acoptolabrus*, *Eupachys*, *Shenocoptolabrus*, *Cathaicus* and *Coptolabrus*. Of these, *Pseudocoptolabrus* and *Megodontoides* seem to be the outgroups of this lineage. Morphological diversification in this lineage is the most remarkable not only within the Procrustimorphi but also throughout the Carabina. Because of this diversity, many (sub)genera have been established as enumerated above. The members of this lineage are rather widely distributed in the Chinese Continent and the nearby regions (Taiwan, Korean Peninsula, Far East, Sakhalin, Japan and the Kurils).

The European lineage

The distributional range of this lineage includes Europe, North Africa and western Asia. Their phylogeny based on morphology has been quite ambiguous especially for some of the members. On the other hand, the results by the molecular phylogeny reveal that the taxonomically established genera are clearly separated from one another on a phylogenetic tree except for *Chrysocarabus olympiae* (Fig. 3).

The sequences of *Macrothorax morbillosus* from Tunisia and Is. Sardinia are almost the same. They are clustered together with the European genus *Chrysocarabus* to which *C. auronitens*, *C. hispanus* and *C. rutilans* belong, although the branching point between the two genera is deep. This suggests that their common ancestry invaded long ago from Europe to North Africa where *Macrothorax* differentiated. The three *Chrysocarabus* species are well separated from each other on the tree.

Two *Imaibius* species (*baronii* and *pachtoun*) from Pakistan form one independent cluster, though their branching point is deep.

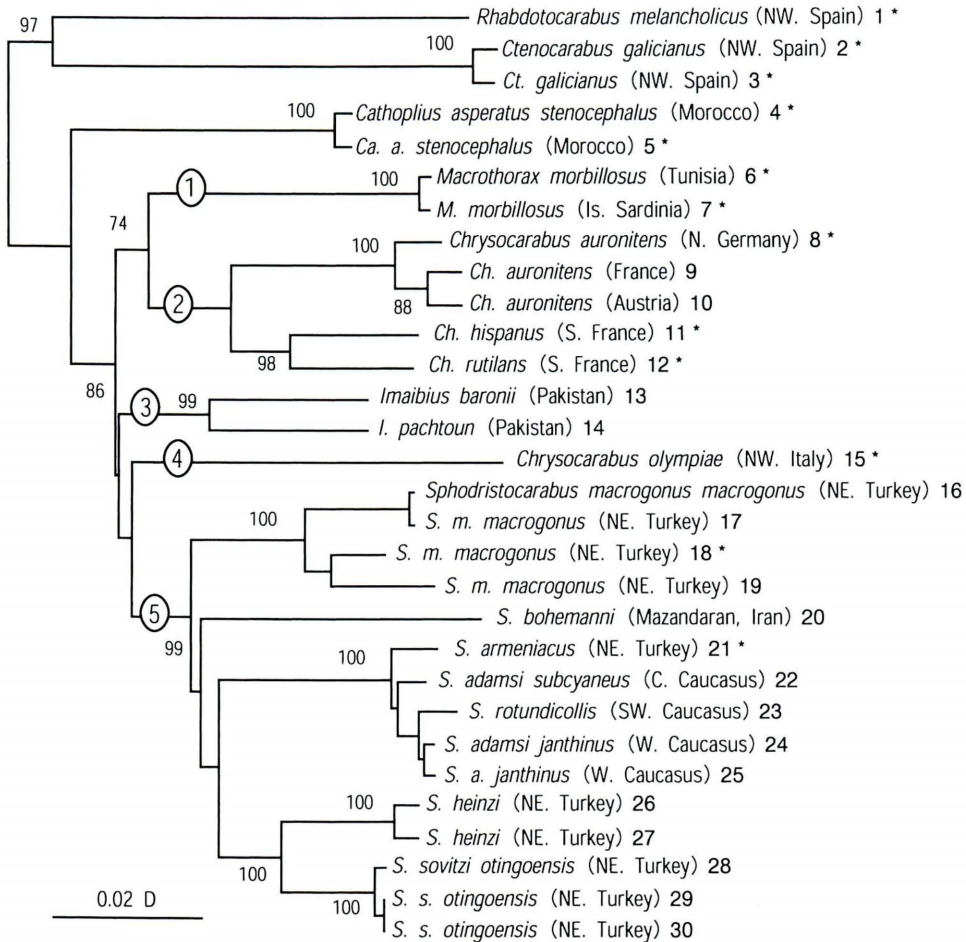


Fig. 3. Phylogenetic tree (NJ) of the mitochondrial ND5 gene of the European lineage.

Unexpectedly, “*Chrysocarabus*” *olympiae* from the alpine region of northwestern Italy appears on the tree at a place entirely independent from that of the *Chrysocarabus* cluster mentioned above. The origin of *olympiae* is old, and perhaps it has been isolated within a limited area of northwestern Italy for a long time.

The genus *Sphodristocarabus* is distributed from Asia Minor to northern Iran, and has been a rather difficult group for taxonomy. The molecular phylogenetic tree reveals that *Sphodristocarabus* is monophyletic, and each species examined forms its own cluster separated clearly from the other species, except that *armeniacus*, *adamsi* and *rotundicollis* form one cluster with only small sequence differences (see below). The first sublineage, the *macrogonus* cluster, is divided into two lines. To the first line belong two specimens from Ordu and the second one is composed of the specimens from

Trabzon and Giresun. Although all these are referable to the nominotypical *macrogonus*, the sequence divergence among each population is not so small. The classification of the *adamsi* complex has been confused, and the taxonomic treatment of the species and/or subspecies has been variable according to the authors. So far as judging from the present molecular phylogenetic tree, however, such lower taxa as *armeniacus*, *adamsi*, *subcyaneus*, *rotundicollis* and *janthinus* are phylogenetically very close to one another.

The Caucasian lineage

This lineage is composed of four genera, *Microplectes*, *Archiplectes*, *Tribax* and *Cechenochilus* distributed mainly in Caucasasia. The latter two are known also from northeastern Turkey. Figure 4 shows the molecular phylogenetic tree of this lineage, which is separated into six clusters.

The cluster 1 consists solely of the members of the genus *Cechenochilus* which are divided into two subclusters with a deep branching point. The first one is represented by *C. heydenianus* from western Caucasus, and the second contains *C. boeberi* from central Caucasus.

The cluster 2 contains a single species, *Microplectes convallium*, and yet it is clearly divided into two lineages each corresponding to two different subspecies. The divergence of these two lineages occurred about 28 million years ago. This suggests that their morphologies have kept almost unchanged at a species level for a long time. The ancient divergence of the two subclusters with a little, at most subspecific, morphological changes may be taken as an example of silent evolution as seen in the *Microplectes* cluster.

The genera *Archiplectes* and *Tribax* appear in the remaining four clusters (the clusters 3 to 6) which are remote from one another on the tree. The cluster 6 further radiated into six subclusters shortly after its separation from other clusters in this lineage. The members of *Archiplectes* belong to the clusters 3 and 5, as well as one of six subclusters within the cluster 6, while the species of *Tribax* belong to the cluster 4 and five different subclusters in the cluster 6. Thus, the separate appearance of both *Tribax* and *Archiplectes* in several independent (sub)clusters would imply that neither of these morphologically defined two genera is monophyletic.

The Eurasian lineage

The principal constituents of this lineage are the genera *Megodontus*, *Procrustes* and *Procerus*. The distributional range, above all that of *Megodontus*, is exceptionally wide in the Procrustimorphi, which covers the greater part of northern Eurasia and northwestern North America. The morphological difference is considerable between the genera, but is not so remarkable within each genus. On the ND5 phylogenetic tree of this lineage (Fig. 5), there are recognized four major clusters, each of which emerged shortly after the radiation of various procrustimorphous lineages.

The cluster 1 is solely composed of the *Megodontus* species, and is further divided into at least three subclusters, each containing 1) two species, *M. exaratus* and

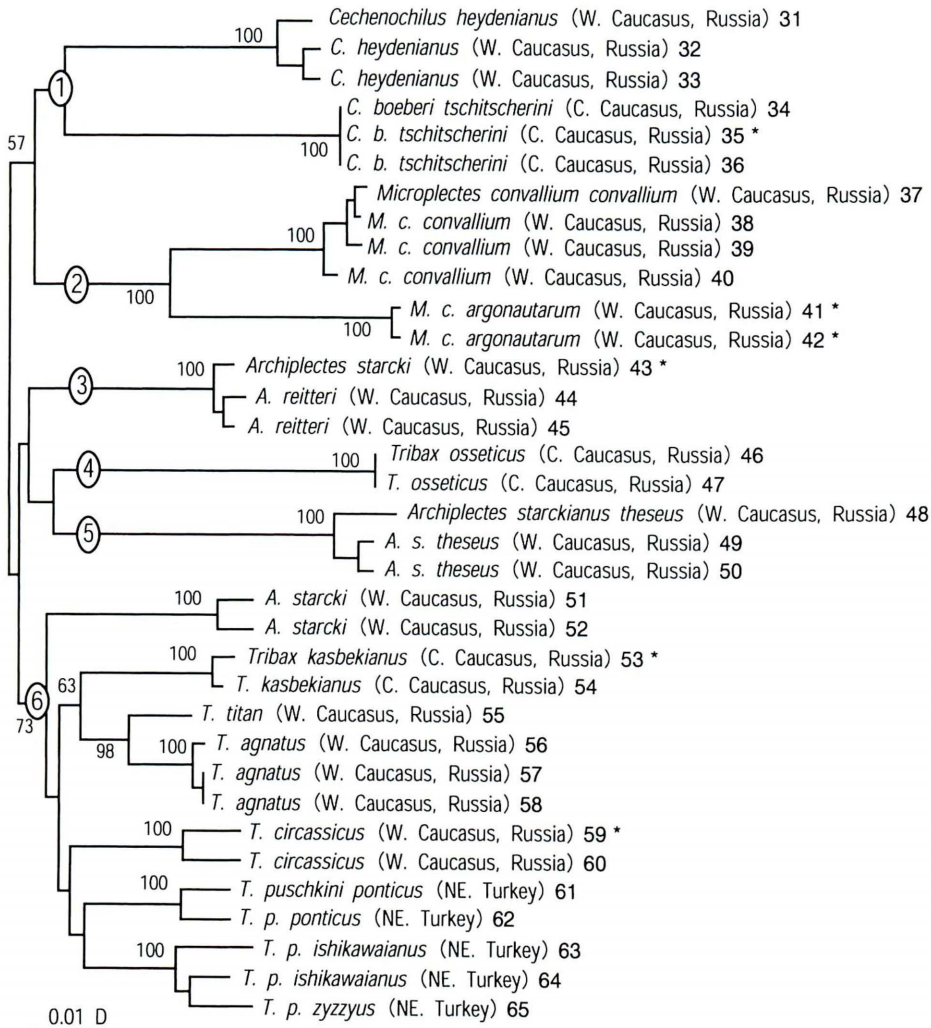
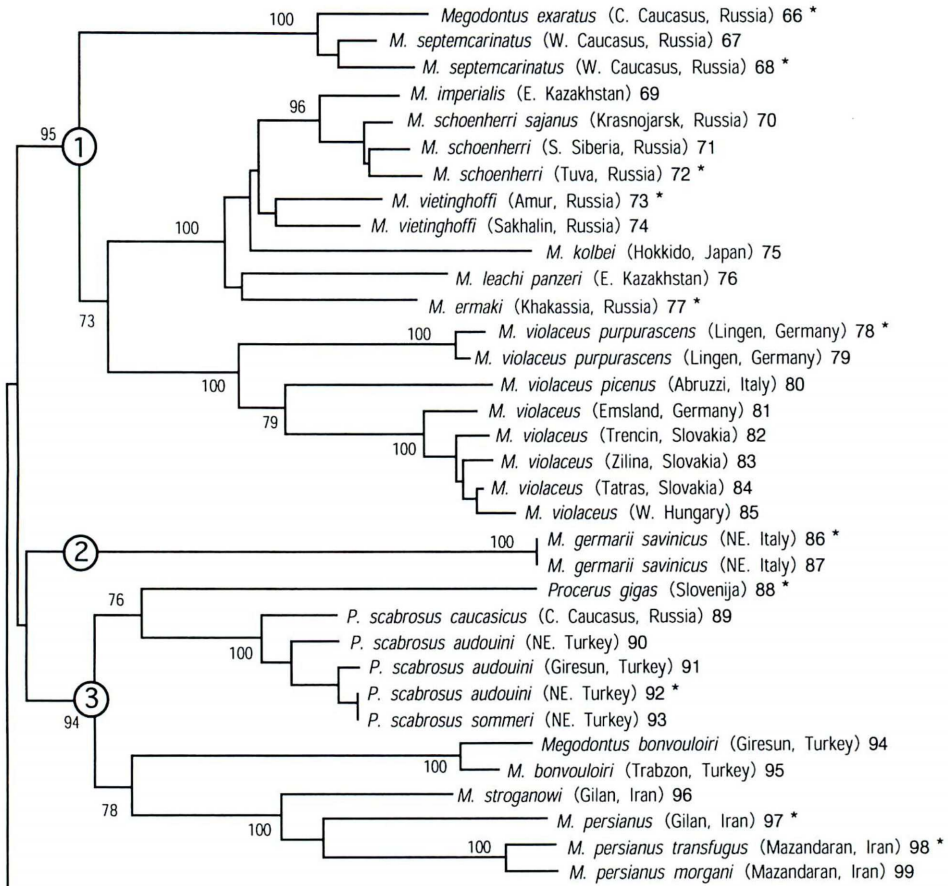


Fig. 4. Phylogenetic tree (NJ) of the mitochondrial ND5 gene of the Caucasian lineage.

M. septemcarinatus from Caucasus, 2) six species from central and eastern Asia and 3) one species (*M. violaceus*) from Europe.

The cluster 2 contains only a single species, *M. germarii* from northeastern Italy, represented by subsp. *savinicus*. The two specimens analysed gave the identical sequence. This species is morphologically very close to *M. violaceus* which belongs to the cluster 1, and has sometimes been treated as one of its local races. However, note that these two taxa are phylogenetically quite remote from each other as shown in Fig. 5.



The cluster 3 is divided into two subclusters. The first one is solely composed of the genus *Procerus*. All the subspecies of *P. scabrosus* analysed here are well separated from *P. gigas*. The second subcluster is composed of three species of *Megodontus* from Turkey and Iran. It is remarkable that these two, traditionally discriminated genera are clustered together.

From these results, it may be inferred that the ancestry of the Eurasian lineage would have been proto-*Megodontus* type, from which *Procerus* branched off. The appearance of *violaceus*/*germarii*-type morphology in two distinct lines may be interpreted in such a way that *germarii* is the ancestral form and its morphology has kept almost unchanged up to the present. An alternative possibility, which is less likely, is that the *violaceus*/*germarii*-like morphology emerged in parallel.

The cluster 4 includes three genera, i.e., *Procrustes*, *Lamprostus* and *Oxycarabus*. The species of *Procrustes* are widely distributed in Europe (except the northern part and the Iberian Peninsula) and Asia Minor to northern Iran, while *Lamprostus* is more

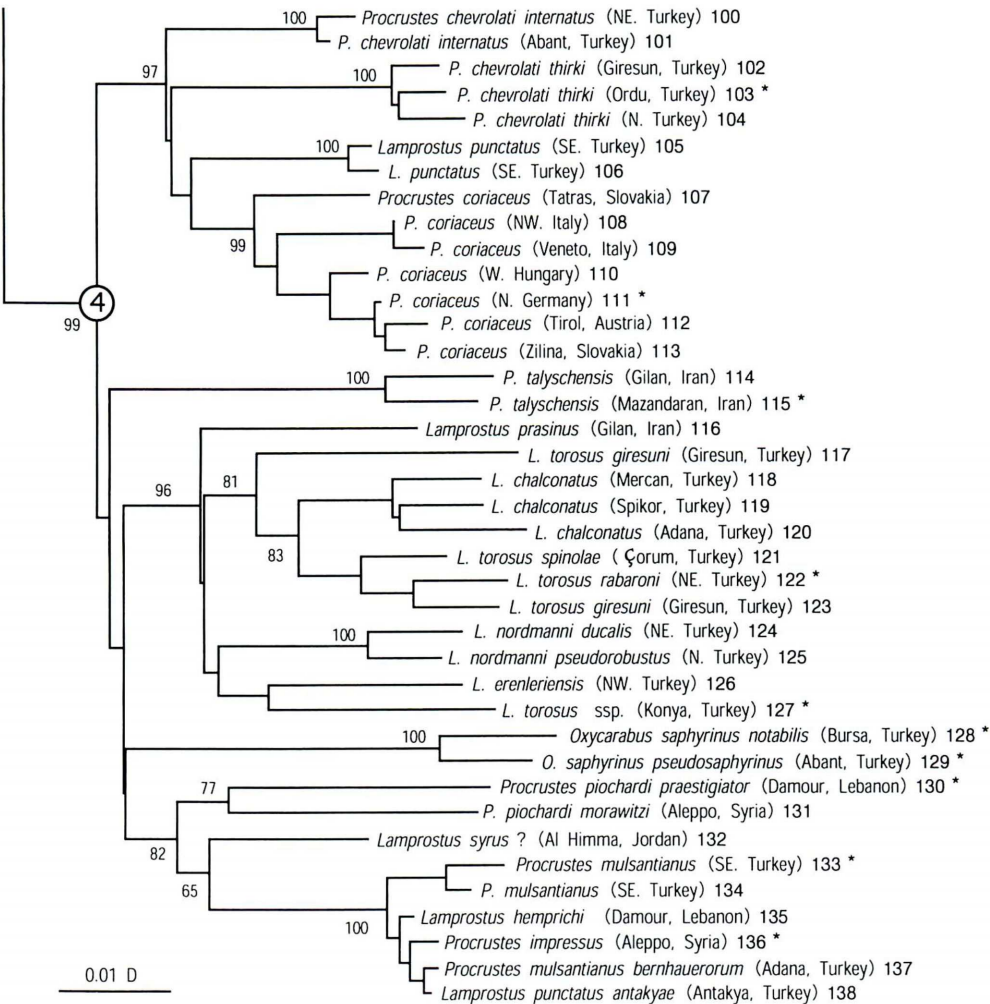


Fig. 5 (on pp. 276–277). Phylogenetic tree (NJ) of the mitochondrial ND5 gene of the Eurasian lineage.

narrowly restricted to Asia Minor. *Oxycarabus* is known only from northern Turkey, and its systematic position has not been well settled by morphology. This cluster is divided into five subclusters, emergences of which seem to have started at about the same time. Since the members of *Procrustes* and *Lamprostus* appear in three distinct subclusters and do not form a single cluster specific to their own, they are clearly polyphyletic. In the first and fourth subclusters, the species of *Procrustes* and *Lamprostus* are intermingled. According to IMURA and MIZUSAWA (1996), *Lamprostus* is very close to *Procrustes* in morphology and may be connected together. From the molecular phylogenetic tree, there is no rationale to separate them, either. It is possible to establish

four distinct genera for the members of *Procrustes* and *Lamprostus* based on the ND5 tree. The diversification of the ND5 gene within the European species, *Procrustes coriaceus*, seems to have started considerably later than that within the Turkish species, suggesting the past invasion of the *P. coriaceus* ancestor (perhaps a proto-form in the first subcluster) from somewhere in Turkey to Europe. The position of *Oxycarabus* is of special interest. This genus is phylogenetically related to *Procrustes*/*Lamprostus*, suggesting that the morphologically specialized *Oxycarabus* line emerged from the restricted area of Turkey as a distinct subcluster within the *Procrustes*/*Lamprostus* cluster.

The Tianshanese lineage

The members of this lineage are distributed in the Tianshan Mountains and mostly macrocephalic. Taxonomy of this group is not consistent with the ND5 phylogeny in many respects. The same species or the same genera are scattered in different clusters on the tree (Fig. 6). There are recognized about ten clusters which seem to have radiated shortly after the radiation of the Carabina. Their exact branching order cannot be estimated by the same reason as for the Caucasian lineage. The clusters 2 to 5 are supported by a node with a high bootstrap value (86%) and were probably derived from the common ancestor.

One specimen of *Cratophyrtus kaufmanni* (referable to subsp. *microcratophyrtus*) from the Tshatkal (=Chatkal) Mountains in Kirghizia (cluster 1) is quite remote from two specimens of the same species (referable to the nominotypical subspecies) from the Turkestan Mountains (cluster 10). The former is clustered with *Pantophyrtus brachypedilus* and *P. turcomannorum*, while the latter two appear with *P. turcomannorum* and *Leptoplesius merzbacheri*, suggesting a parallel appearance of *Cratophyrtus* and *Pantophyrtus* in two different lines. Similarly, the members of *Cratocephalus* separately appear in three different clusters (clusters 4, 5 and 8). *Cratocechenus akinini* appears in the clusters 2 and 3, and *Eotribax* in the clusters 2 and 6. The clusters 3 (*Cratocechenus*), 7 (*Deroplectes*) and 9 (*Alipaster*) are each composed of a single genus, and the latter two do not appear in other clusters.

The Chinese lineage

The Chinese lineage consists of more than 20 genera containing over 100 species and many subspecies. In spite of the morphological diversity, this lineage forms a group on the ND5 tree separated from the other procrustimorphous lineages (Fig. 1). According to the molecular phylogenetic tree of the Chinese lineage (Fig. 7), we tentatively assume that eight groups (clusters 1 to 8) radiated within a relatively short period upon diversification of this lineage. Each cluster is respectively referable to the following genera: 1) *Pseudocoptolabrus*, 2) *Megodontoides*, 3) *Acathaicus*, 4) *Coptolabrodes*, 5) *Imaibiodes*/*Lasiocoptolabrus*/*Aristocarabus*/*Shunichiocarabus*/*Pagocarabus*/*Neoplesius*/*Pseudocranion*/*Eccoptolabrus*/*Calocarabus*/*Eocechenus*, 6) *Damaster*, 7) *Cephalornis* and 8) *Acoptolabrus*/*Eupachys*/*Shenocoptolabrus*/*Cathaiacus*/*Coptolabrus*. For *Neoplesius*, *Damaster*, *Acoptolabrus* and *Coptolabrus*, many

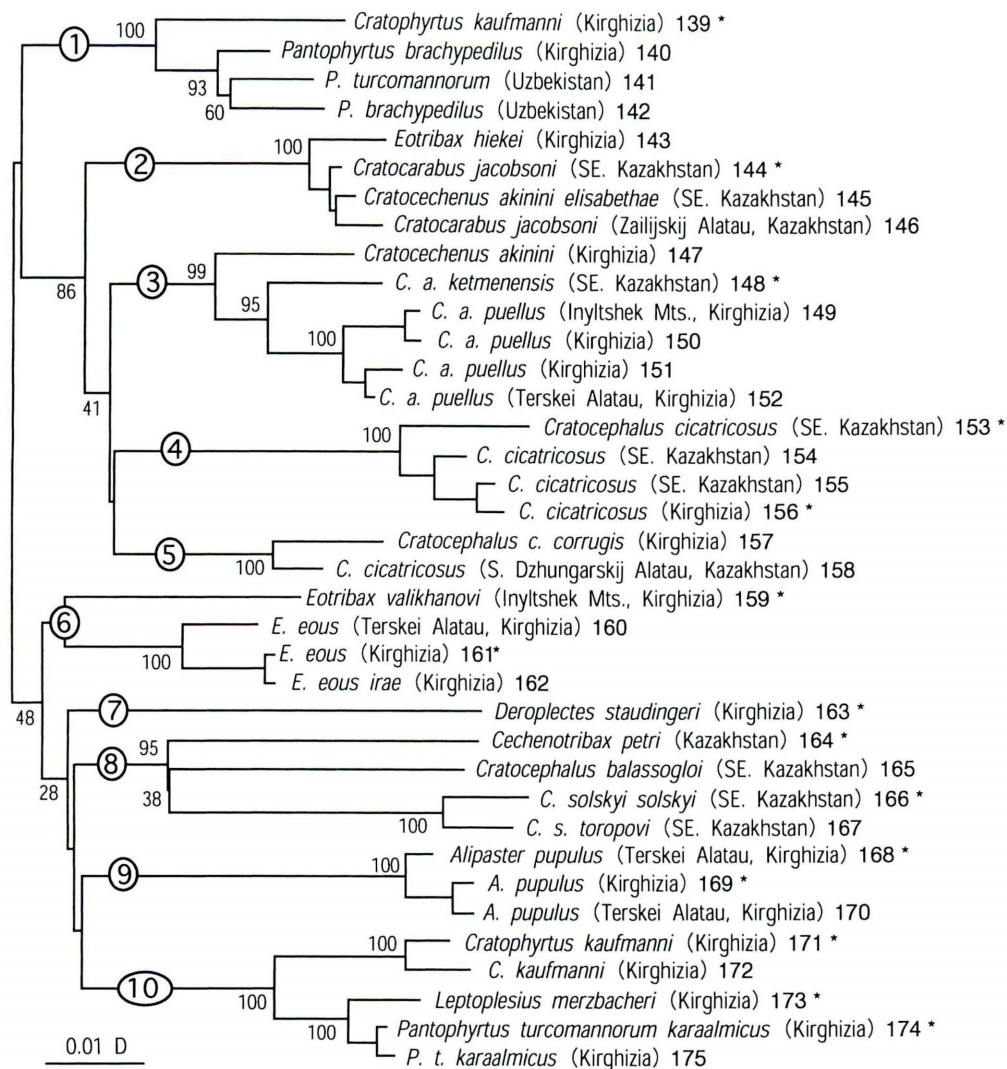
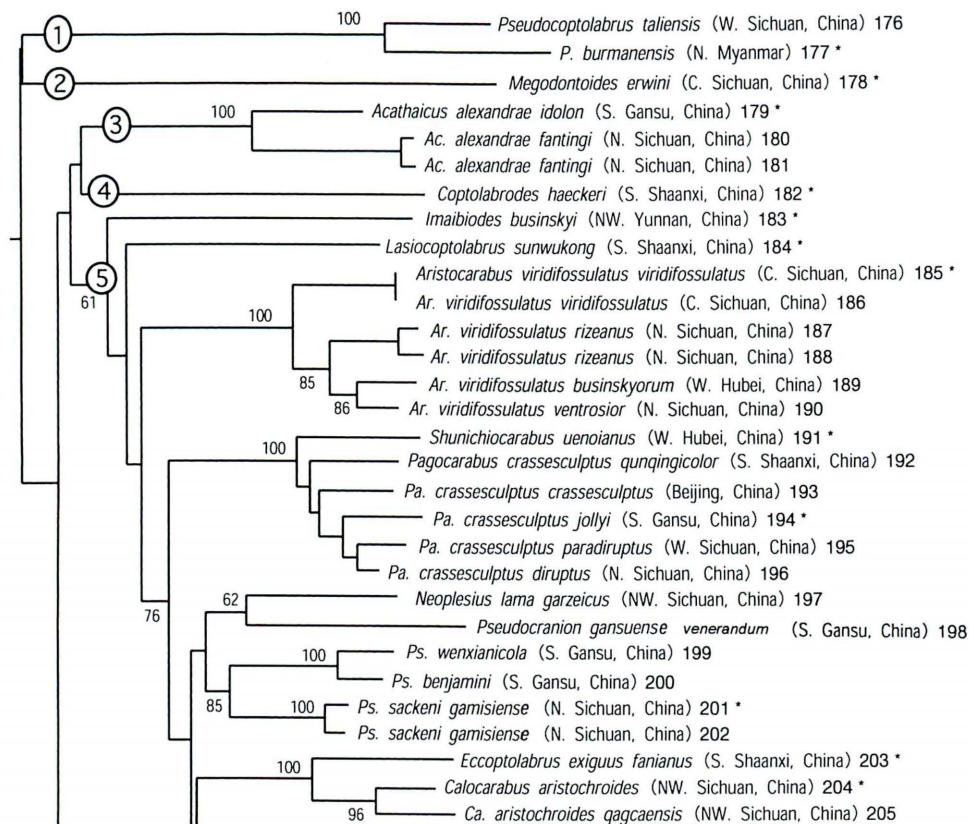


Fig. 6. Phylogenetic tree (NJ) of the mitochondrial ND5 gene of the Tianshanese lineage.

more specimens (nearly 200) have been analysed. To avoid complexity, however, only a limited number of them were used for Fig. 7. For the details, see IMURA *et al.* (1998) for *Neoplesius* and the allied genera, SU *et al.* (1998) and KIM *et al.* (1999) for *Damaster*, OKAMOTO *et al.* (in preparation) for *Acoptolabus/Coptolabus*. *Pseudocoptolabus* (cluster 1; distributed in northern Myanmar, northwestern Yunnan and southwestern Sichuan) and *Megodontoides* (cluster 2; central Sichuan) are somewhat remote from other Chinese groups, and seem to have diverged a little earlier than the others. It is



possible, though not certain, that either one of their proto-forms is the ancestry of the whole Chinese lineage. Because of the extreme morphological diversity, estimation of the phylogenetic relationships of this lineage is almost hopeless from the cladistic analysis based on morphology alone. In short, the ND5 phylogenetic tree reveals that in many cases morphology does not reflect phylogeny, and the important principles governing the evolution of the carabid beetles are exemplified in this lineage.

Morphological similarity does not necessarily reflect phylogenetic relatedness — Parallel evolution

The genera *Damaster* (s. str.), *Coptolabrus* and *Acoptolabrus* have often been incorporated into *Damaster* (s. lat.) from the morphological viewpoint (ISHIKAWA, 1986, '91, etc.). In addition to these three, the genera *Coptolabrodes* and *Shenocoptolabrus*, both of which have been recently described from China, are suggestive of an apparent affinity to *Damaster* (s. lat.) (BŘEZINA & IMURA, 1997; IMURA *et al.*, 1999). *Coptolabrodes* is morphologically very similar to *Acoptolabrus*, and *Shenocoptolabrus* has morphological characters of *Acoptolabrus*, *Coptolabrus* and *Damaster* combined to-

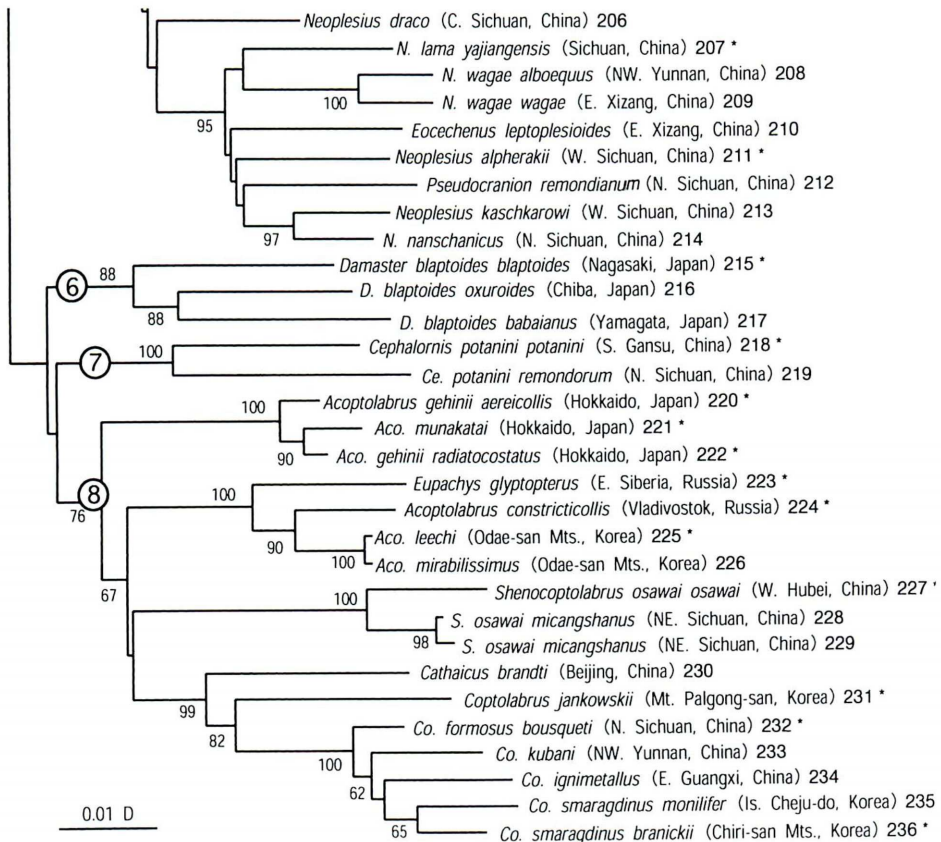


Fig. 7 (on pp. 280–281). Phylogenetic tree (NJ) of the mitochondrial ND5 gene of the Chinese lineage.

gether. However, as shown in Fig. 7 (clusters 4, 6 and 8), there is no firm evidence for these five genera to have a direct phylogenetic relatedness. Rather, the emergence of each of them is old so as to form its independent cluster on the tree (for *Damaster* and *Acoptolabus*, see below). From the cladistic analysis based on morphology, it was assumed that *Damaster* (s. str.) shares the common ancestry with *Coptolabus* (ISHIKAWA, 1986). Later, SU *et al.* (1996 b) assumed that the ancestor of *Damaster* (s. str.) would be *Acoptolabus* rather than *Coptolabus* by examining the ND5 gene sequences only from a few Japanese species. However, the present molecular analyses using much richer materials from the Chinese Continent strongly suggest the weak relatedness of *Damaster* (s. str.) either to *Coptolabus* or to *Acoptolabus*. The genera *Eupachys*, *Acathaicus* and *Cathaicus* are morphologically similar, all in having the blackish stout body with remarkable macrocephalism, and have been thought to be phylogenetically closely related to one another. The ND5 tree reveals the absence of any phylogenetic relatedness among them (see below). *Neoplesius lama garzeicus* and

N. draco are remote from the other *Neoplesius* species. The examples mentioned above suggest that parallel morphological evolution has occasionally taken place.

Morphological dissimilarity does not necessarily reflect phylogenetic unrelatedness

Two macrocephalic species, *Eupachys glyptopterus* and *Cathaicus brandti* (see above), are respectively clustered on the ND5 tree with *Acoptolabrus* spp. and *Coptolabrus* spp., both of which are beautifully decorated and morphologically quite different from the macrocephalic species. The clustering of a macrocephalic species, *Eocechenus leptoplesioides*, with the *Neoplesius* spp. (mostly non-macrocephalic) has been reported already (IMURA *et al.*, 1998). In the present study, it has become apparent that two *Pseudocranion* species are clustered with the *Neoplesius* species. *Pseudocranion gansuense* was coupled with *N. lama garzeicus* and *P. remondianum* appeared in the major *Neoplesius* cluster. The genus *Calocarabus* consists of several beautifully decorated species distributed in the high mountains of western China, and has been placed near *Eocechenus* and *Neoplesius*. The genus *Eccoptolabrus* is composed of a single species, *exiguus*, known from Shaanxi, Gansu and Sichuan, and has been positioned near *Lasiocoptolabrus* and *Coptolabrus*. External morphology of *Calocarabus* is quite different from that of *Eccoptolabrus*, and yet they are clustered together on the ND5 tree. *Shunichiocarabus* and *Pagocarabus* are morphologically distinct at a generic level and yet they are closely related on the ND5 tree.

Radiation and discontinuous evolution

As mentioned above, not a few groups of the Chinese lineage radiated after its emergence. The radiation and the occurrence of morphologically dissimilar genera (or species) within a single cluster (see the preceding section) suggest that morphological evolution is discontinuous (see SU *et al.*, 2001). This in turn suggests the presence of silent periods with little morphological changes. This may be estimated by comparisons of morphological changes with the time elapsed after diversification of the ND5 sequences within the same or the closely related species. Many examples of such a silent evolution can be recognized by a large evolutionary distance between the geographically separated same or allied species such as *Damaster blaptoides* (SU *et al.*, 1998), *Cephalornis potanini*, *Acathaicus alexandrae* (Fig. 7), *Neoplesius* spp. (IMURA *et al.*, 1998) and others.

Taxonomic notes

From the phylogenetic analyses presented above, the taxonomic reorganization of the Procrustimorphi should obviously be required. Such an attempt, named "classification of the subtribe Carabina based on molecular phylogeny", has been made by IMURA (2002) using mainly the phylogenetic trees of the ND5 gene presented in this paper. However, it must be pointed out that at the present time there is no absolutely ideal way to classify a given organismic group, and yet IMURA's attempt has given us one of the reasonable taxonomic arrangements of the procrustimorphous ground beetles which we believe to be much more objective than those performed by morphological

knowledge alone.

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要 約

金 衝坤・蘇 智慧・井村有希・岡本宗裕・大澤省三：ミトコンドリアND5遺伝子の塩基配列からみた世界のヨロイオサムシ群の系統と進化。—— オサムシ亜族中，最大のグループであるヨロイオサムシ群Procrustimorphiに属する123種236個体について，ミトコンドリアND5遺伝子の塩基配列を決定し，分子系統樹を作成したところ，本群は分布域と密接に関連した5系統（ヨーロッパ系，コーカサス系，ユーラシア系，天山山脈系，中国系）に分れることが判明した。これら5系統それぞれの詳しい系統樹を示し，ヨロイオサムシ群内の系統関係と進化の様式について考察を加えた。

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